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PRINCIPAL INVESTIGATOR: Dr. Kai Leung

CONTRACTING ORGANIZATION: The Geneva Foundation
Tacoma, WA 98402

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Dr. Kai Leung
email: kai.p.leung.civ@mail.mil

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The FDA-approved drug pirfenidone is an anti-inflammatory/anti-fibrosis drug indicated for pulmonary fibrosis that we hypothesize can diminish scarring when applied topically to deep partial-thickness burn wounds in two animal models. The long-term objective is to learn to effectively use pirfenidone with regard to dosage, formulation and timing of treatment of burn wounds, such that animal studies will likely translate to the clinic. The objective of this proposal is to evaluate pirfenidone for efficacy in reducing fibrosis and scarring parameters in mouse and porcine models of deep partial-thickness burn wounds. The dosage formulation and schedule of treatment will be optimized and molecular markers of inflammation, angiogenesis, wound healing, and fibrosis will be correlated with scar reduction.

15. SUBJECT TERMS None listed

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INTRODUCTION

Deep Partial-thickness (DPT) burns frequently result in hypertrophic scars that can lead to severe functional impairment, psychological morbidity, and costly long term healthcare. Current treatment options lack effectiveness. The purpose of this research is to identify dosage formulations and treatment schedules for the FDA-approved drug Pf to evaluate it for use as a topical prophylactic and treatment against fibrotic scarring of DPT-burn wounds. The scope of the research is to evaluate pirfenidone for efficacy in reducing fibrosis and scarring parameters in mouse and porcine models of deep partial-thickness burn wounds. The dosage formulation and schedule of treatment will be optimized and molecular markers of inflammation, angiogenesis, wound healing, and fibrosis will be correlated with scar reduction.

KEYWORDS

Deep Partial-Thickness Burn; Pirfenidone; Hypertrophic Scar; Fibrosis; Formulations; mouse Burn Model; Porcine Burn Model; Topical; Inflammation; Granulation; Proliferation

ACCOMPLISHMENTS

What were the major goals of the project?

1. Identification of topical formulations and doses that effectively deliver Pf to the dermis of DPT-burn wounds at each phase of healing and mitigate fibrosis of the closed wounds.
2. Optimization of the schedule of topical applications and uses this optimized schedule to determine detailed molecular changes in healing wounds resulting from Pf treatment.
3. Validation of the efficacy of Pf to reduce hypertrophic scarring in the Duroc porcine DPT-burn model.

What was accomplished under these goals?

The followings summarize the major activities and accomplishments associated with the goals described above for Year 2:

Major accomplishments of Goal #1:

Identified topical formulations and doses that effectively deliver Pf to the dermis of DPT-burn wounds at each phase of healing and mitigate fibrosis of the closed wounds.

Formulation of Pirfenidone (PF) Ointment

For dose-finding of PF to reduce inflammation and fibrosis after deep partial-thickness burn, we prepared three PF ointment formulations at 1, 3.5, and 6.5% (w/w) (Table 1) and analyzed their PF release profiles. More than half of PF (50-60%) was released from these ointments in the first 24h, and most remaining PF was released by 48h (Fig. 1). Additionally, these ointments were stable in color, appearance, and drug content during storage at 25^{o}C/60% RH for 3 months (data not shown) (Dorati et al., 2017). Thus, these ointment formulations are well-suited for topical delivery of PF to wounds.

Table 1. Composition of PFD ointment formulations

Formulations	Pirfenidone	Ointment base	Mineral oil	Polyethylene glycol	Benzyl alcohol
F1[a]	1	78	20	-	
F2[a]	3.5	70.5	20	5	1
F3[a]	6.5	90	2.5	-	1

[a] Batch size – 50 g

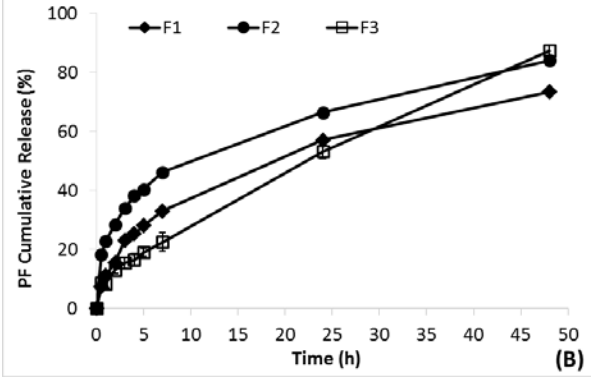


Figure 1. Cumulative PFD release *vs.* incubation time for three different formulations containing PFD at 1 (F1), 3.5 (F2), and 6.5 (F3) %, respectively. PFD was released from the ointments (250 mg) into phosphate-buffered saline, pH 6.8, at 34˚C (n=3) in a Franz diffusion cell (Hanson Research Corp.) and was assayed by HPLC (Waters™) with an Inertsil ODS-2 column and 310 nm detection against a PFD standard curve (3-30 µg/mL).

Pirfenidone-Loaded Microparticles

Spray drying was used to produce pirfenidone (PF)-loaded microparticles for the sustained release of pirfenidone over a period of 7 days for use in the treatment of porcine deep partial-thickness burn wounds. PLGA polymers based on different composition and Mw have been used to prepare spray dried microparticles. In our third quarter progress report, we described the use of RG 502H (PLGA 50:50, Mw 24-38 KDa) to prepare PF-loaded microparticles, which showed an unwanted rapid burst release. As an alternative approach, we prepared other PF-loaded microparticles using a different Mw PLGA, Resomer RG 753 H (PLGA 75:25) polymer instead. Different amounts of PF (2.5, 5, 10 and 20 % w/v) were successfully encapsulated into PLGA microparticles by spray drying. Table 2 summarizes the experimental conditions used for preparing PF-loaded microparticles.

Table 2. PF loaded microparticles prepared by spray drying technique using Resomer RG 753 H

Batch #	Polymer type	Terminal group	Mw (kDa)	Polymer conc in DMC (w/w %)	Theoretical loading (%)	Air Speed (M^3/min)	Air in Temp (˚C)	Pump rate (g/min)	Process Yield (%)
5	PLGA 75:25	Uncapped	12.9-21.1	2.4	-	0.3	RT	7-8	45.09
6	PLGA 75:25	Uncapped	12.9-21.1	2.4	2.5	0.3	RT	7-8	46.22
7	PLGA 75:25	Uncapped	12.9-21.1	2.68	5	0.3	RT	12-15	51.54
8	PLGA 75:25	Uncapped	12.9-21.1	2.83	10	0.3	RT	12-15	51.46
9	PLGA 75:25	Uncapped	12.9-21.1	2.91	20	0.3	RT	12-15	65.24

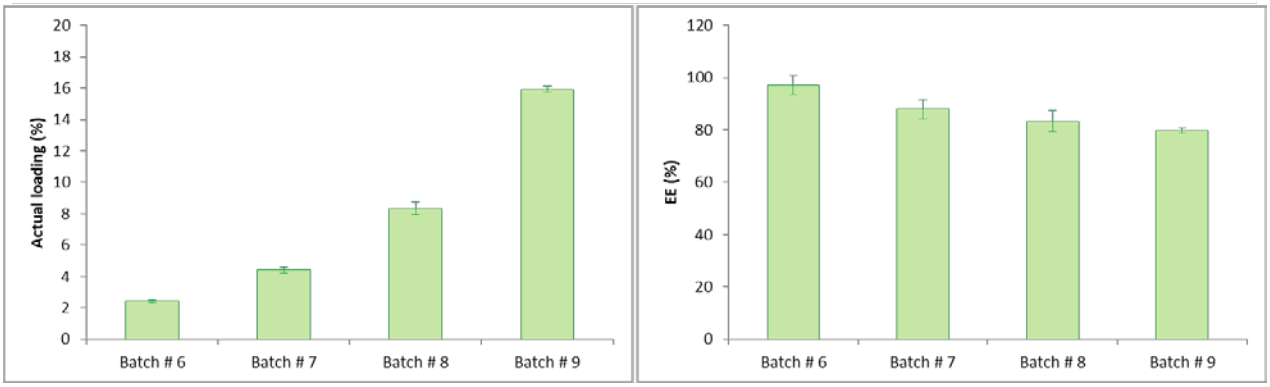


Figure 2. Actual loading and encapsulation efficiency (EE) percentages of PF loaded microparticles (Batch # 6-9). Actual loading corresponds to mg of PF in 100 mg of microparticles, while EE is the percentage of PF encapsulated into microparticles respect with the theoretical loading (amount of PF weighted for preparation of microparticles batch).

The encapsulation efficiency of PF-loaded microparticles prepared was >79 % (Figure 2). However, *in vitro* release of PF-loaded microparticles showed a burst release of >70% of PF within 6 hours (Figure 3), which was similar to that of PF-loaded microparticles prepared using RG 502H (data shown in Q3Y2 quarterly report). We will continue to optimize the preparation of PF-loaded microparticles for extended release without the unwanted early burst release. Different experimental parameters such as changing the size of nozzle, polymer concentration, and solvent used to dissolve polymer will be tested to optimize the release.

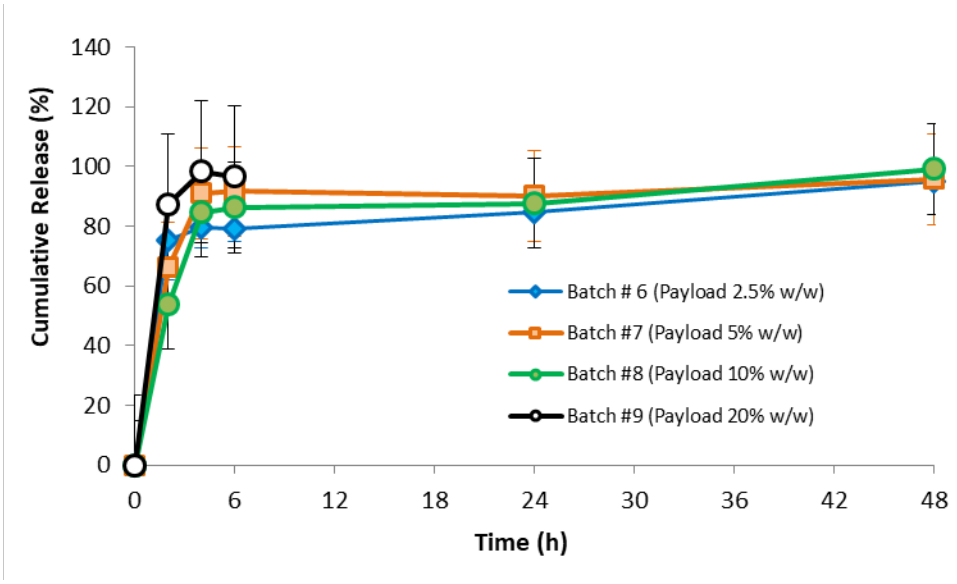


Figure 3. Effect of different payload on *in vitro* release of PF from PLGA microparticles based on PLGA 75:25 (Mw 24-38 kDa). Batches prepared by spray drying.

Pirfenidone-loaded Polylactic-co-Glycolic Acid (PLGA) Films

The objective of this study is to develop PLGA films that can deliver the pirfenidone (PF) over 7 days for burn wound healing application. Briefly, PLGA-based films were fabricated by a solvent casting technique. Polymer solution was prepared by dissolving PLGA polymer in methylene chloride (DCM). Different additives including polyethylene glycol (PEG) and glycerol (GLY) were added to the polymer solution to enhance the elasticity of the films. The dissolved mixture was transferred into a silicon mold and then dried at room temperature for 72 hours. The composition of the polymers, plasticizers, and drugs used for the film preparation is summarized in Table 3.

Table 3. The effects of addition of varying amount of plasticizers on PF release profiles *in vitro*.

Film #	PLGA (%, w/v)***	PF (% w/v)****	Plasticizer (%, w/v)	
			PEG 400	Glycerol
1	12.5	0.625	-	-
2	12.5	0.625	2	0.2
3	12.5	0.625	2	0.4
4	12.5	0.625	2	0.8
5	12.5	0.625	3	0.2
6	12.5	0.625	3	0.4
7	12.5	0.625	3	0.8
8	12.5	0.625	4	0.2
9	12.5	0.625	4	0.4
10	12.5	0.625	4	0.8

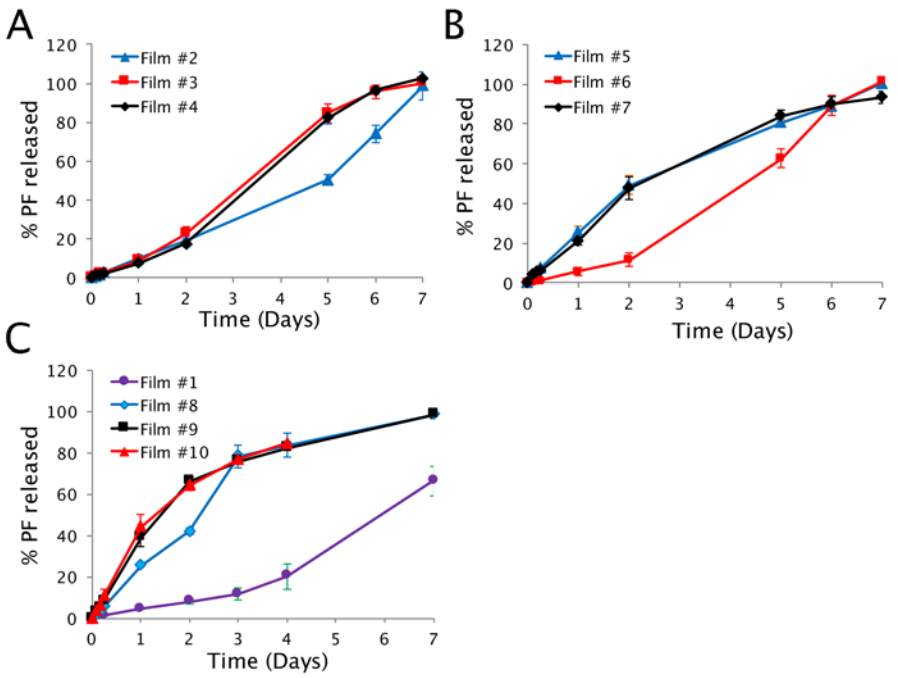


Figure 4. **Cumulative release of pirfenidone from PLGA films.** *In vitro* release study was performed by incubating each film in 7 mL PBS (pH 6.8) at 34 ˚C. At predetermined time point, the samples (1 mL) were collected and replaced with fresh buffer. Percent PF released was determined by UV-vis at 310 nm.

Figure 4 (A-C) shows the release profiles of PF from PLGA films with various concentrations of plasticizers and PEG400. PF was completely released from the test films in 7 days. The results showed that plasticizers affected the release profiles of PF (Figure 4A-C). Additionally, the inclusion of plasticizers caused a reduction of glass transition temperature, resulting in an increased elasticity of the films. With 4% PEG400, we were able to obtain the 7 day release of PF with a 30-40% burst release after 1 day. This study demonstrates that films #8, 9, and 10 are promising candidates as the PF-releasing wound dressing that can provide extended release of the antifibrotic drug over a period of 7 days. Further studies are in progress to prepare films with addition of various adhesive excipients and to their test mechanical properties, including tensile testing, shear stress, peel adhesion, and tack testing.

Mechanism(s) of action of Pirfenidone

Reduction of the Profibrotic Phenotype of Human Dermal Fibroblasts

Pirfenidone treatment of cultured normal human dermal fibroblasts (NHFD) stimulated with TGF-β1 reduced their migration and proliferation dose-dependently. PFD also reduced the profibrotic phenotype of TGF-β1-stimulated human dermal myofibroblasts, decreasing the amount of alpha smooth muscle actin (α-SMA), collagens, stress fibers, and focal adhesions (Fig. 5), and the phosphorylation of p38 kinases (Fig. 6). Furthermore, in these cells, PFD downregulated the expression of α-2 smooth muscle actin (*ACTA2*), connective tissue growth factor (*CTGF*), matrix metalloproteinase 1 (*MMP1*), and other markers of fibrosis (data not shown) (Hall, Wells, and Leung, 2017). These results together with published studies suggest that PDF inhibits p38 and other MAP kinases involved in inflammation and fibrosis.

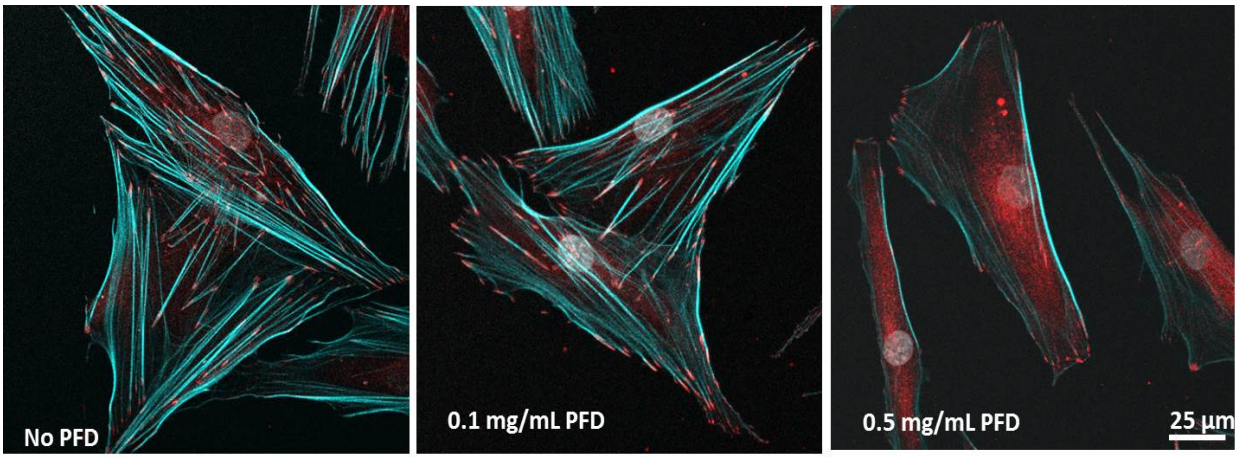


Figure 5. Pirfenidone (PFD) alters formation of focal adhesions (red punctate foci) and stress fibers (turquoise) in TGF β1 stimulated human dermal fibroblasts (NHDF). NHDF seeded at 2,000 cells/cm^2 were fixed after serum starvation and 5 days of treatment in serum-free media (SFM) with 10 ng/mL TGF-β1, TGF-β1 and 0.1 mg/ml or 0.5mg/mL PF, or SFM alone Cells were fixed in paraformaldehyde and stained with Alexa Fluor 647-conjugated Phalloidin (1:40) for actin filaments, Hoec 33342 (1:2000) for nuclei (white round bodies), and anti-Vinculin mouse mAb (1:300) detected with Alexa Fluor 568-conjuga goat anti-mouse secondary antibody (1:1000) for focal adhesions. Note the stress fibers (actin filaments containing non-muscl myosin) anchoring into focal adhesions that are located in the specialized regions of the cell membrane.

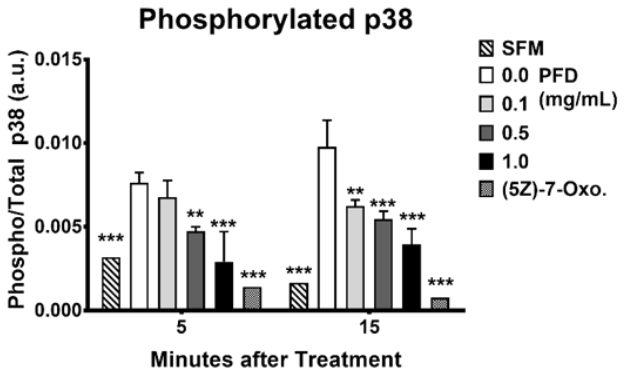


Figure 6. PFD suppressed p38-mediated MAPK but not SMAD2/3 signaling. NHDF cells stimulated with TGF-β1 alone or with PFD were analyzed for p38 and SMAD2/3 phosphorylation (western blots). The mean phosphorylated protein levels ± s.d. for three replicate experiments are shown. ***P<0.01*, ****P<0.001*: TGF-β1 alone vs. with PFD. The TAK1/p38 inhibitor, 5(Z)-7-oxozeaneol (10 µM), reduced phosphorylation of p38 and SMAD2/3 to a similar extent as PFD.

Modulation of the Inflammatory Profiles of Human Neutrophils (UTSA subaward)

Isolated human neutrophils were stimulated with different agonists: lipopolysaccharides-LPS, fMet-Leu-Phe-fMLP, or High Mobility Group Box 1-HMGB1. Pirfenidone treatment of stimulated human neutrophils increased expression of CD66b (marker for neutrophil activation-degranulation) (Fig. 7) and collagen degradation (Fig. 8), reduced production of reactive oxygen species (ROS) (Fig. 9), and decreased phagocytosis (Fig. 10). Pirfenidone also increased apoptosis of neutrophils stimulated with the damage-associated molecular patterns, HMGB1, but not other agonists (Fig. 11). These results suggest that pirfenidone regulates both pro- and anti-inflammatory responses in neutrophils *in vitro*.

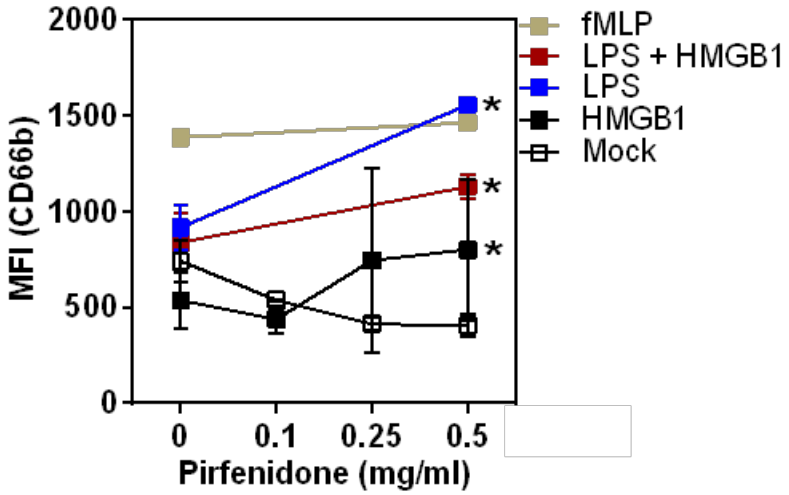


Figure 7. Neutrophil degranulation. Neutrophils, stimulated with agonists for 4 hours, were treated with pirfenidone at different concentrations. Cells were stained for CD66b using specific mouse anti-CD66b antibodies and quantified by flow cytometry. The experiment was performed in triplicate showing the standard deviation ± standard error; * $p \leq 0.05$, student's t-test for significant difference in comparison to no treatment with drug. LPS, 100 ng/ml; fMLP, 100 nM; and HMGB1, 100 ng/ml. MFI, Mean Fluorescence Intensity.

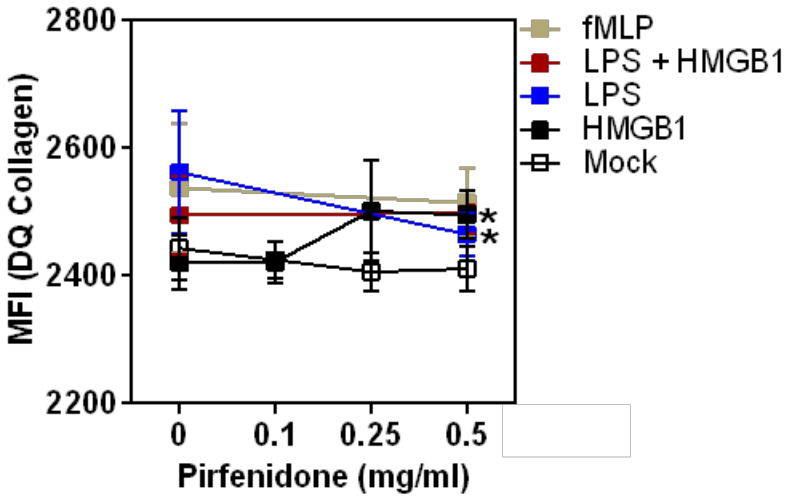


Figure 8. Neutrophil Collagen degradation. Neutrophils were stimulated with agonists and treated with pirfenidone at different concentrations for 4 hours concurrently. Collagen degradation was measured using DQ collagen as the substrate in a fluorescent plate reader. Degradation of DQ collagen caused emittance of fluorescence. Fluorescence was measured using a plate reader. The

experiment was performed in triplicate showing standard deviation ± standard error; * p≤ 0.05, student's t-test for significant difference in comparison to no treatment with drug. LPS, 100 ng/ml; fMLP, 100 nM; and HMGB1, 100 ng/ml. MFI, Mean Fluorescence Intensity.

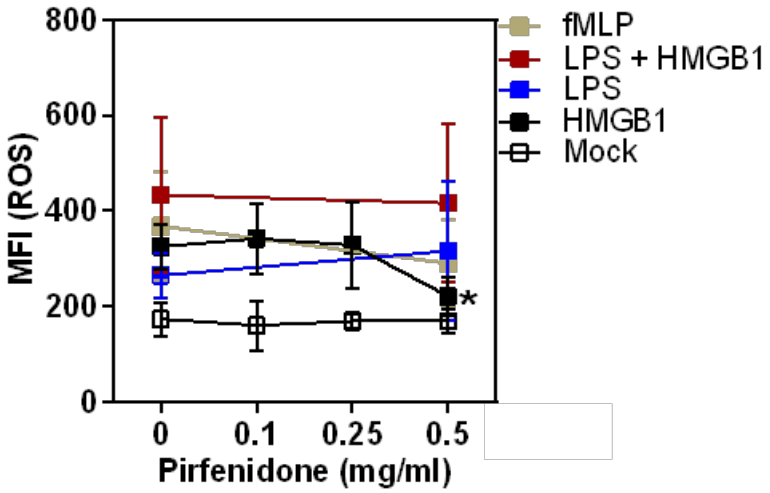


Figure 9. Neutrophil ROS production. Neutrophils were stimulated with agonists and treated with pirfenidone at different concentrations for 4 hours concurrently. ROS (reactive oxygen species) production from treated neutrophils was determined using a commercially available ROS/SO (superoxide) detection kit. Fluorescence emitted as the results of oxidation of the fluorescent probes by ROS produced by the treated cells was measured using a plate reader. The experiment was performed in triplicate showing the standard deviation ± standard error; * p≤ 0.05, student's t-test for significant difference in comparison to no treatment with drug. LPS, 100 ng/ml; fMLP, 100 nM; and HMGB1, 100 ng/ml. MFI, Mean Fluorescence Intensity.

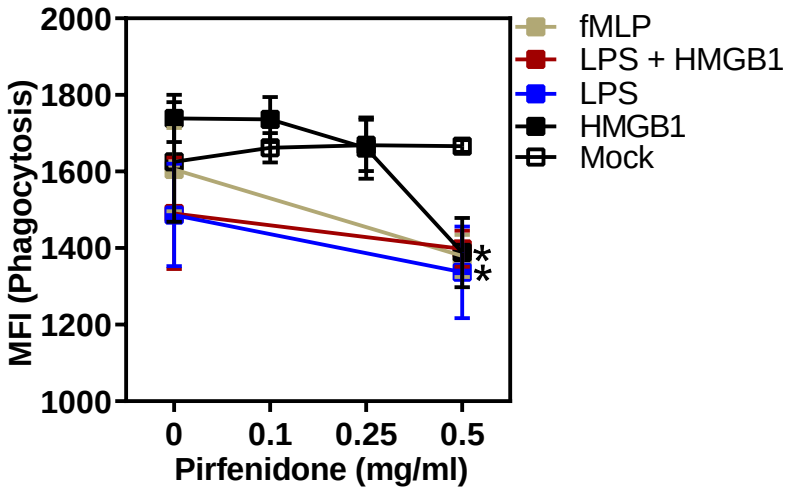


Figure 10. Neutrophil phagocytosis. Neutrophils were stimulated with agonists and treated with pirfenidone at different concentrations for 4 hours concurrently. Phagocytosis was measured using the pHrodo-labeled Green *Escherichia coli* Bioparticles. Low pH in phagosomes caused increased fluorescence from ingested labeled *E.coli.* Fluorescence was measured using a plate reader. The experiment was performed in triplicate showing the standard deviation ± standard error; * p≤ 0.05, student's t-test for significant difference in comparison to no treatment with drug. LPS, 100 ng/ml; fMLP, 100 nM; and HMGB1, 100 ng/ml. MFI, Mean Fluorescence Intensity.

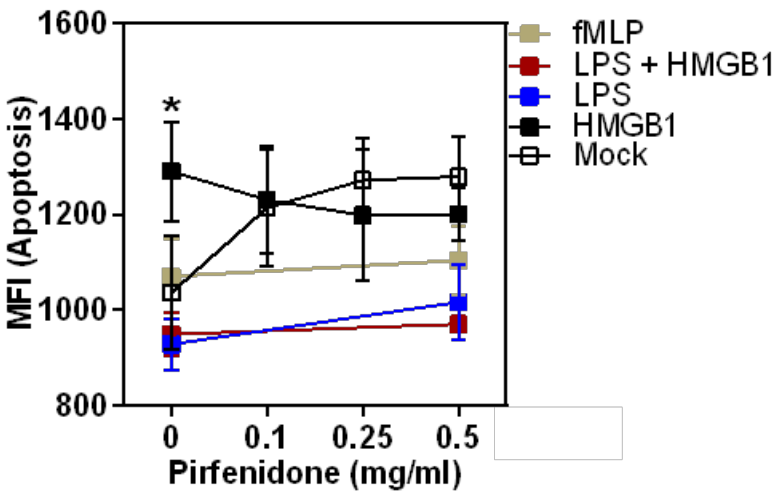


Figure 11. Neutrophil apoptosis/necrosis. Neutrophils were stimulated with agonists and treated with pirfenidone at different concentrations for 4 hours concurrently. Neutrophil apoptosis and necrosis was measured using a commercially available detection kit (Abcam) to label cells at late stage apoptosis and necrosis (with resultant loss of plasma membrane integrity) with a membrane-impermeable fluorescent dye. Fluorescence emitted from the labeled cell nucleus was measured using a fluorescence plate reader. The experiment was performed in triplicate showing the standard deviation ± standard error; * $p \leq 0.05$, student's t-test for significant difference in comparison to no treatment with drug. LPS, 100 ng/ml; fMLP, 100 nM; and HMGB1, 100 ng/ml. MFI, Mean Fluorescence Intensity.

Major accomplishments of Goal #2:

Optimization of the schedule of topical applications and uses this optimized schedule to determine detailed molecular changes in healing wounds resulting from Pf treatment.

Treatment of deep partial-thickness mouse burn

Topical application of the 6.5% PDF ointment to mouse deep partial-thickness burn wounds (Medina et al. 2017) during the inflammatory phase (2 treatments, days 0 and 2 post-burn) of healing decreased α-SMA (Figure 12), reduced inflammatory cytokines (TNF-α, IL-2, IL-12p70, IL-13) (Figure 13), and lessened skin fibrosis (Figure 14), but did not delay wound closure (data not shown). Additionally, treatment of burn wounds during the remodeling phase, but not the proliferative phase, showed a trend in reducing skin fibrosis (Figure 14). The results suggest that optimal treatment schedule of pirfenidone ointment to reduce fibrosis of mouse deep partial-thickness burn wounds occurs during the inflammatory and remodeling phases, but not during the proliferative phase of healing.

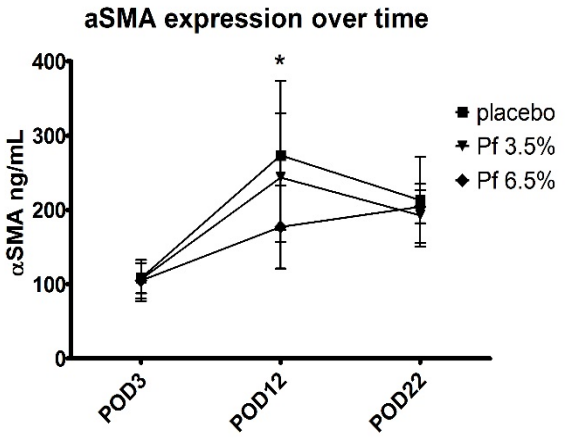


Figure 12. PFD ointment applied topically to deep partial-thickness burn wounds reduced α-SMA protein expression (ELISA). Mouse burn wounds were treated with PFD twice, immediately and at 48 hrs post-burn. α-SMA in wound skin homogenates was assayed by ELISA. α-SMA protein was significantly lower in mice treated with 6.5% PFD vs. ointment vehicle (2-way ANOVA; mean -/+ std) on day 12. * $p<0.05$.

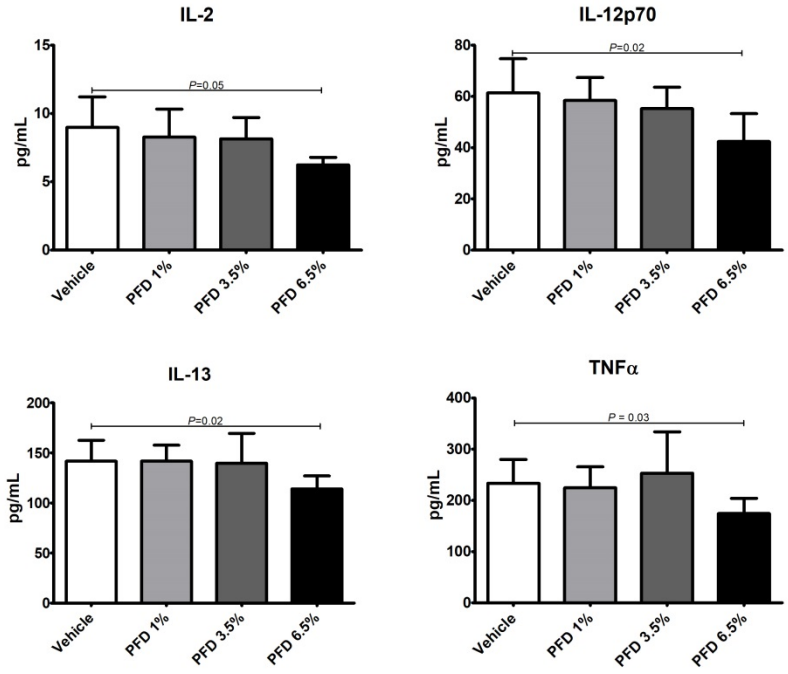


Figure 13. PFD ointment applied topically to deep partial-thickness burn wounds reduced IL-2, IL-12p70, IL-13, and TNF-α. Mouse burn wounds were treated with PFD twice, immediately and at 48 hrs post-burn. Inflammatory cytokines in wound skin homogenates were assayed by ELISA. This early treatment during the inflammatory stage of healing significantly reduced inflammatory cytokines of the burn tissue at 22 days post-burn *vs.* ointment vehicle ($p<0.05$; 2-way ANOVA; mean -/+ std). Vehicle, ointment without PFD.

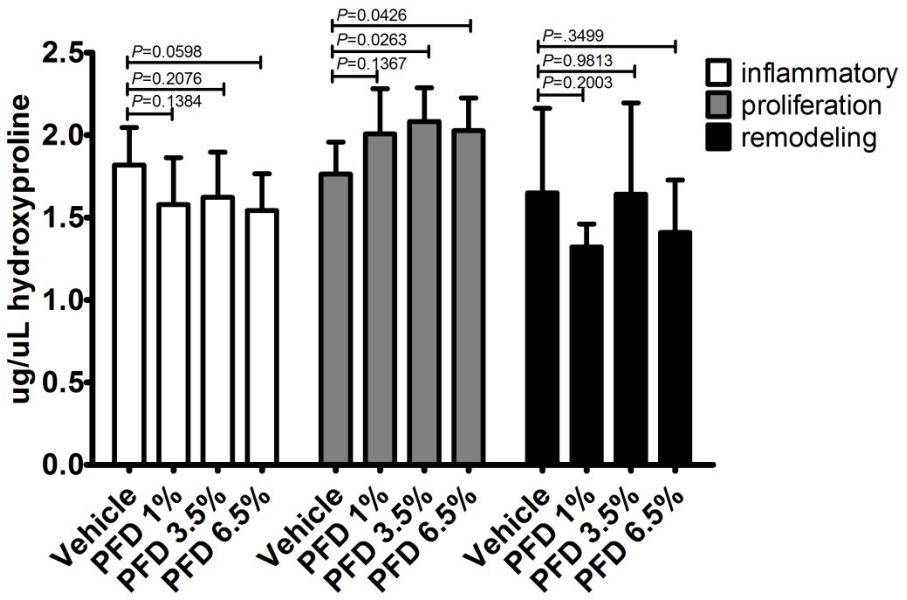


Figure 14. The fibrosis indicator, hydroxyproline, a measure of collagen content, quantified at 22 days post-burn. Mouse burn wounds were treated with PFD during 3 phases of healing post-burn: Days 0 and 2 (inflammatory phase), Days 4, 6, 8, and 10 (proliferative phase), or Days 14, 16, 18, and 20 (remodeling phase). Compared to the ointment vehicle, treatments during the inflammatory and remodeling phases, but not the proliferative phase, show a trend in collagen reduction. Hydroxyproline is expressed µg per µl of hydrolyzed wound tissue. Vehicle, ointment without PFD.

References

Dorati, R, J. L. Medina, P. P. DeLuca and K. P. Leung. Development of an Ointment Formulation as an Antiscarring Treatment for Deep Partial-Thickness Burn. 2017. *Submitted (Pharm Sci Tech).*

Hall, C. L, A. R. Wells, and K. P. Leung. Pirfenidone Reduces Profibrotic Responses in Human Dermal Myofibroblasts *In Vitro.* 2017. *Submitted (Laboratory Investigation).*

Medina, J. L., A. Fourcaudot, E. Sebastian, R. Shankar, A. Brown, and K. P. Leung. Standardization of Deep Partial-Thickness Scald Burns in C57BL/6 Mice. 2017. *Submitted (Burn).*

What opportunities for training and professional development has the project provided?

Nothing to Report.

How were the results disseminated to communities of interest?

We intend to publish the findings in a peer-reviewed journal as a means to disseminate the results to reach the members of research communities who are interested in developing therapeutic solutions to reduce

fibrosis and scarring. Recently, we have submitted three manuscripts describing the mouse burn model, effects of pirfenidone on human dermal fibroblasts, and ointment formulations of pirfenidone, respectively, to peer reviewed journals for consideration of publication (see below for authors and title of the submitted manuscripts).

What do you plan to do during the next reporting period to accomplish the goals?

We plan to do the following in the next reporting period:

1. Test the treatment effect of mouse DPT burn wounds for reduction of fibrosis by combining pirfenidone treatments during both inflammatory (days 0 and 2 post-burn) and remodeling (days 14, 16, 18, and 20) phases of healing.

2. Continue to develop pirfenidone microspheres and films for sustained release of pirfenidone for up to 7 days.

3. Develop the porcine deep partial-thickness burn model to test the efficacy of formulated pirfenidone to reduce scarring.

IMPACT

What was the impact on the development of the principal discipline(s) of the project?

The mouse data obtained provided some insights on the treatment schedule as to when the topical pirfenidone ointment applied at the appropriate time to have the beneficial anti-fibrotic effect.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

CHANGES/PROBLEMS

Changes in approach and reasons for change

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report.

PRODUCTS

Publications, conference papers, and presentations

Journal publication.

Dorati, R, J. L. Medina, P. P. DeLuca and K. P. Leung. Development of an Ointment Formulation as an Antiscarring Treatment for Deep Partial-Thickness Burn. 2017. *Submitted (Pharm Sci Tech).*

Hall, C. L, A. R. Wells, and K. P. Leung. Pirfenidone Reduces Profibrotic Responses in Human Dermal Myofibroblasts *In Vitro.* 2017. *Submitted (Laboratory Investigation).*

Medina, J. L., A. Fourcaudot, E. Sebastian, R. Shankar, A. Brown, and K. P. Leung. Standardization of Deep Partial-Thickness Scald Burns in C57BL/6 Mice. 2017. *Submitted (Burn).*

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers, and presentations.

Oral Presentation (Caroline Hall), February 16, 2017
Hall, CL; Wells, AR; Leung, KP
"Pirfenidone reduces the profibrotic response in an in vitro model of TGF-beta1-induced human dermal myofibroblasts"
RegenMedSA (San Antonio Conference on Stem Cell Research & Regenerative Medicine)

Poster Presentation (Caroline Hall), June 16, 2017
Hall, CL; Wells, AR; Leung, KP
"Pirfenidone reduces the profibrotic response in an in vitro model of TGF-beta1-induced human dermal myofibroblasts"
SURF (San Antonio Military Health System and Universities Research Forum)

Poster Presentation (Caroline Hall), August 29, 2017
Hall, CL; Wells, AR; Leung, KP
"Pirfenidone Reduces Profibrotic Responses in Human Dermal Fibroblasts"
MHSRS (Military Health System Research Symposium), Orlando, FL

Poster Presentation (Adrienne Wells), August 29, 2017
Wells, AR; Hall, CL; Leung, KP
"Anti-fibrotic Pirfenidone Modulates Mechanoresponsive Machinery in

Myofibroblasts"
MHSRS (Military Health System Research Symposium), Orlando, FL

Oral & Poster Presentation (Caroline Hall), September 21, 2017 **1st Place Poster Presentation
Hall, CL; Wells, AR; Leung, KP
"Pirfenidone Reduces Profibrotic Responses in Human Dermal Fibroblasts"
SAPRF (San Antonio Postdoctoral Research Forum)

Poster Presentation (Adrienne Wells), September 21, 2017 **2nd Place Poster Presentation
Wells, AR; Hall, CL; Leung, KP
"Anti-fibrotic Pirfenidone Modulates Mechanoresponsive Machinery in Myofibroblasts"
SAPRF (San Antonio Postdoctoral Research Forum)

Website(s) or other Internet site(s)

Nothing to report.

Technologies or techniques
Nothing to report.

Inventions, patent applications, and/or licenses

Materials were submitted to MRMC for consideration of a Provisional Patent Application for the utility of pirfenidone as a topical prophylactic anti-scarring treatment of deep partial-thickness burns.

Other Products

Nothing to report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Kai Leung
Project Role:	PI
Nearest person month worked:	3
Contribution to Project:	Dr. Leung is responsible for insuring compliance with all regulatory requirements. He has chosen the following personnel to assist him in the proposed studies because of their expertise in animal surgery and in the field of molecular biology, histology, histochemistry, and immunohistochemistry, PCR array analysis, as well as wound healing analysis.

Name:	Rodney Chan
Project Role:	Co-PI
Nearest person month worked:	1
Contribution to Project:	Dr. Chan meets with the laboratory staff regularly to discuss the progress of the project, participate in data analysis, and prepare reports and manuscripts.

Name:	Li-Wu Qian
Project Role:	Research Associate
Nearest person month worked:	10.8
Contribution to Project:	Dr. Qian is responsible for the animal surgical procedures and will plan and execute the animal model required for this proposed research. He will be responsible for the animals for insuring compliance with all regulatory requirements for this project and our institution.

Name:	Rossella Dorati
Project Role:	Visiting Formulation Scientist
Nearest person month worked:	12
Contribution to Project:	Dr. Dorati prepares Pirfenidone-loaded microspheres for controlled drug release used in the treatment of deep partial-thickness burn wounds to reduce fibrosis and hypertrophic scarring in mouse and porcine model, respectively. Dr. Dorati also prepares pirfenidone is different dosage forms (cream, gel, and ointment) for use in the treatment experiments.

Name:	Jorge Medina
Project Role:	Post-Doctoral Fellow
Nearest person month worked:	9.6
Contribution to Project:	Dr. Medina develops the deep partial-thickness mouse burn model for use in testing the treatment of burn wounds containing pirfenidone to reduce fibrosis in mouse.

Name:	Sooneon Bae
Project Role:	Post-Doctoral Fellow
Nearest person month worked:	6.0
Contribution to Project:	Dr. Bae prepares Pirfenidone-loaded films for use in testing the treatment of burn wounds containing pirfenidone to reduce hypertrophic scarring in a porcine deep partial-thickness burn model.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Not applicable

Provide the following information for each partnership:

Organization Name:

Not applicable.

Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- Financial support;
- In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);
- Facilities (e.g., project staff use the partner's facilities for project activities);
- Collaboration (e.g., partner's staff work with project staff on the project);

 Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and
 Other

SPECIAL REPORTING REQUIREMENTS

Quad Chart: *The Quad Chart (available on https://www.usamraa.army.mil) shall be updated and submitted as an appendix.*

APPENDICES

Quad Chart is included.

“Anti-scar Treatment for Deep Partial-thickness Burn Wounds”

Log Number: BA150467

Award Number: W81XWH-15-2-0083

PI: Dr. Kai Leung **Org:** The Geneva Foundation/USAISR **Award Amount:** $2,177,795

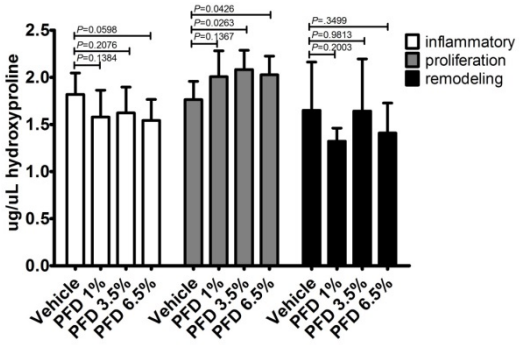
Study/Product Aim(s)

- Aim 1: Identify topical formulations and doses that effectively deliver pirfenidone (Pf) to the dermis of deep partial-thickness (DPT) burn wounds at each phase of healing and mitigate fibrosis of closed wounds.
- Aim 2: Optimize the schedule of topical applications and use this optimized schedule to determine detailed molecular changes in healing wounds resulting from Pf treatment.
- Aim 3: Validate the efficacy of Pf to reduce hypertrophic scarring in the Duroc porcine DPT-burn model.

Approach

As much as half or more of DPT burn wounds develop hypertrophic scarring, and once formed, treatments are only minimally effective. The proposed research seeks to re-purpose the FDA-approved drug Pf for use as an anti-scarring treatment for DPT-burns. Re-purposing builds upon previous R&D, making the regulatory path shorter. The proposed R&D aims to learn to use Pf with regard to dosage, formulation, and timing of treatment of burn wounds, and molecular markers, such that animal studies will likely translate to the clinic. This information would support advanced development of Pf as an anti-scarring treatment.

Pirfenidone showed a trend in reducing fibrosis in mouse DPT burn wounds



Accomplishments: (1) Formulated Pf-containing films with promising controlled & extended release profiles;(2) Pf treatments targeted p38 kinases and reduced expression of key fibrosis-associated genes and production of collagen in TGF beta-stimulated human dermal fibroblasts; (3) Pf treatments during the inflammatory and remodeling, but not proliferative phase, showed a trend in reduction of fibrosis in mouse DPT burn wounds.

Timeline and Cost

Activities CY	15	16	17	18
Identify formulations that penetrate tissue and mitigate fibrosis indicators.				TRL2
Optimize the schedule of topical applications and identify anti-fibrosis markers.				TRL3
Validate the efficacy of Pf to reduce hypertrophic scarring in the Duroc porcine DPT-burn model.			TRL4	
Estimated Budget ($2.17M)	**171,885**	**702,189**	**745,632**	**558,090**

Updated: October 17, 2017

Goals/Milestones

FY16 Goals

- ❑ Topical dosage formulation of Pf that delivers the drug to the dermis at each phase of healing as determined by pharmacodynamic effects and anti-fibrosis indicators in the mouse DPT-burn model.

FY17 Goals

- ❑ Optimal formulated dose and schedule of topical application of Pf that best diminishes scarring endpoints in the mouse DPT-burn model.

FY18 Goals

- ❑ Determination of the effectiveness of the optimal formulated Pf dose and schedule of topical application that reduce hypertrophic scarring in the Duroc porcine DPT-burn model.

Purpose: To provide proof-of concept for the repurposing of the FDA approved anti-fibrotic drug Pirfenidone as a prophylactic to reduce scar caused by DPT burns.

Product: An FDA approved prophylactic anti-scar agent .

Payoff: To improve scarring outcomes of deep partial-thickness burn.

Budget Expenditure as of 9.30.17

Projected Expenditure: $1,619,706

Actual Expenditure: $338,915